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Effect of Diet on Copper Toxicity to 'Neanthes arenaceodentata' (Annelida: Polychaeta)

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Effect of diet on copper toxicity to Neanthes arenaceodentata (Annelida: Polychaeta)*

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16. ABSTRACT

Groups of Neanthes arenaceodentata were fed different diets for four weeks prior to exposure to copper in seawater to see if nutritional history would affect toxicity. Diets used were: a combination of cultured fresh Ectocarpus siliculosis and Tetra Marin, rehydrated field-collected and dried Enteromorpha clathrata, and fresh cultured Enteromorpha. Copper levels in food were determined after 48 hours in the exposure system. Diet had an effect on the short term results (4- to 10-day LC50 values) but not on long term results (28 day LC50 values).

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Abstract

Groups of Neanthes arenaceodentata were fed different diets for four weeks then exposed to copper in seawater to determine if nutritional history would affect copper toxicity. Two experiments were conducted in which mortality was the endpoint. In the first experiment, the 28-day LC50 concentrations were >98 µg/L Cu for polychaetes fed a combination diet of cultured fresh Ectocarpus siliculosus and TetraMarin, 80 µg/L Cu for those fed field-collected dried Enteromorpha clathrata (fed rehydrated), and 38 µg/L Cu for those fed cultured fresh Enteromorpha clathrata. The greater sensitivity of worms fed the fresh E. clathrata may have resulted because the fresh E. clathrata accumulated Cu while in the experimental system, therefore, worms fed this diet were probably also exposed to Cu through the food. Metal analysis of the food revealed that the mean Cu concentration of fresh E. clathrata was 5 to 6 times higher than the mean Cu concentration of dried E. clathrata or TetraMarin after 48 hours in any of the Cu dosed treatments. Mean Cu concentration of the fresh E. siliculosus was 3 to 5 times higher than dried E. clathrata or TetraMarin after 48 hours in the experimental system. However, it is uncertain whether worms fed the combination diet of E. siliculosus and TetraMarin were affected by the Cu in the fresh alga, because much of the alga was used for tube construction. It is not known how much alga the worms actually consumed. Therefore, in the second experiment, live alga diets and combination diets were not used to avoid these problems.

In the second experiment, diet had an effect on the short-term results (4 to 10-day LC50 values), but not the long-term results (28-day LC50 values). Worms fed prawn flakes were significantly more resistant

to Cu than worms fed dried E. clathrata, the 10-day LC50 values were 246 and 124 µg/L Cu, respectively. The 28-day LC50 values for these two diets were not significantly different (83 and 86 µg/L Cu). The short-term LC50 values in experiment two (where diet-toxicant was minimized) differed by less than a factor of 2, which is within the range of variability (1.18 to 3.31) in an interlaboratory comparison with this species [1].

Key words: copper, toxicity test, aquatic toxicology, polychaetes, Neanthes arenaceodentata, diets

Introduction

The nutrition of test animals is an important factor in any biological experiment. A committee from the Institute of Laboratory Animals Resources, in a report to the National Academy of Sciences [2] stated that nutrition is a major experimental variable. This has been demonstrated in several toxicology studies. Mehrle et al. [3, 4] and Phillips and Buhler [5] have shown that the diet of rainbow trout prior to and during chronic toxicity tests can affect their response to various toxicants. Winner et al. [6] found that Daphnia magna fed vitamin-enriched algae were less sensitive to a chronic copper stress than animals fed a trout ration. Bengtson et al. [7] showed that Atlantic silversides fed different geographic strains of Artemia (known to differ in nutritional quality) yielded significantly different results in acute toxicity tests with cadmium.

The polychaete Neanthes arenaceodentata Moore (Nereis acuminata Ehlers, Nereis caudata delle Chiaje) has been widely used in marine toxicological research [8, 9, 10, 11, 12, 13, 14, 15, and others]. An interlaboratory comparison with N. arenaceodentata has been completed for the U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances [1]. Also, a test system has been developed with the chromosomes of this polychaete to study the effects of genetic toxicants in the marine environment [16, 17, 18]. Recent experiments conducted in our laboratory (Schauer and Pesch, personal communication) demonstrated markedly different growth of N. arenaceodentata with a number of different diets; however, the effect of nutrition on the toxicological response of N. arenaceodentata has not been evaluated.

This report describes selected details of the diet studies with N. arenaceodentata (the full report will be published elsewhere) and the effect of diet on the toxicity of copper to N. arenaceodentata. Two toxicity experiments were conducted with a total of four different diets. In both of these experiments, larvae were fed the diets for about four weeks, then the worms were exposed to Cu in seawater.

Materials and Methods

Diet experiment:

The purpose of this experiment was to screen a large number (19) of diets, however, only five of those diets were used in the subsequent toxicity tests and will be discussed here. These diets included two commercially prepared diets, the fish flake diet TetraMarin (TetraWerke, Dr. rer. nat. Ulrich Baensch GmbH, D4520 Melle 1, West Germany) and prawn flakes (ADT-Prime, Aquatic Diet Technology, Inc., Brooklyn, NY), and three algal diets, cultured brown alga Ectocarpus siliculosus (fed fresh), cultured green alga Entermomorpha clathrata (fed fresh), and field-collected E. clathrata (dried and fed rehydrated).

Five 2-week-old N. arenaceodentata larvae were placed in each Carolina dish (90 mm diameter), with five replicate dishes per diet. Worms were fed three times weekly for 28 days. Water (50 ml sand-filtered Narragansett Bay water, 32 °/oo salinity, 20 ± 1°C) in the dishes was changed prior to each feeding. All treatments were supplied with an equal dry weight of diet (weight of fresh diets was adjusted for moisture content). Because the worms use a portion of the food to construct tubes, we made no attempt to equalize the coloric content of the diets. The amount of food provided

was based on the results of two preliminary tests. The amount of food added to each dish was adjusted for the number of surviving worms. An initial feeding of 3 mg food/worm gave the worms extra material to establish tubes. Worms were then fed the equivalent of 1 mg (dry) food/worm/day until day 19 when the amount of food was increased to 2 mg/worm/day and maintained at this level until the end of the experiment.

The number of worms and tubes were counted at every feeding. Tube production (mean number of tubes per worm) was calculated by averaging the tube to worm ratio for days 12, 21, and 28. At the end of the experiment, worms were removed, oven-dried at 75°C for 24 h, and weighed to the nearest 1.0 µg on a Perkin-Elmer Autobalance* (Model AD-2Z).

Toxicity experiments:

In the first experiment, groups of N. arenaceodentata larvae were fed one of three diets for four weeks, then each group was exposed to three concentrations of Cu or seawater control for 53 days. The diets were (1) a combination of cultured brown alga Ectocarpus siliculosus (fed fresh) and the commercial fish flake diet TetraMarin, (2) cultured green alga Enteromorpha clathrata (fed fresh) and (3) field-collected E. clathrata (dried and rehydrated). These diets were chosen based on the results of the diet experiment.

In the second experiment, groups of N. arenaceodentata larvae were fed one of two diets for four weeks, then each group was exposed to one of four concentrations of Cu or seawater control for 28 days. The diets were (1) commercially prepared prawn flake diet (ADT-Prime*, Aquatic

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Diet Technology, Inc., Brooklyn, New York) and (2) field-collected E. clathrata (dried and rehydrated). These diets were chosen based on the results of our diet experiment and also on the results of the first toxicity experiment.

In the environment, N. arenaceodentata constructs one or several connnecting mucus-lined burrows in the sediment. However, in the laboratory, these animals can be cultured without sediment [15]; they use food and fecal pellets to construct a mucus-lined tube. Because of the impact of sediment on Cu toxicity with this species [14], we conducted one experiment without sediment ani the second experiment with a minimal amount of sediment (see below).

The larvae used in both experiments were obtained from D.J. Reish, California State University. Each batch was shipped shcrtly after the larvae had left the parent tube and started to eat, 28 and 21 days after egg laying, for experiments 1 and 2, respectively. For each experiment a random sample of larvae was selected to determine initial dry weights. These groups of larvae (19 and 25 for experiments 1 and 2, respectively), were oven-dried (75°C for 24 hours), and weighed on a Perkin-Elmer Autobalance* (Model AD-2Z). The remaining larvae were placed in fiberglass trays (43.5 X 62.5 X 7cm, 14L volume) for the feeding portion of the experiment. The incoming water was turned off for several hours, flow rates were adjusted to 25 mℓ/min for the next 24 hours to allow the larvae to settle on the bottom, then flow rates were set at 300 mℓ/min for the remainder of the test.

In the first experiment, larvae from three broods were pooled, then distributed among three trays (about 200 larvae per tray, 1 tray per diet).

The animals were fed three times weekly for 30 days. The amount of food was adjusted so there was minimal excess food. In the second experiment, larvae from five broods were pooled, then distributed among four trays (about 300 larvae per tray, two trays per diet). The animals were fed twice weekly for 27 days; 2.5 gm (dry weight) of food was given per tray for the first feeding and 1.0 gm per tray was fed for the rest of the feedings. This amount resulted in some excess food. Both diets were fed in powdered form (particle size < 180 μm).

At the end of the feeding portion of both experiments, the worms fed the different diets were different sizes. This size difference was apparent visually; however, worms in all diets appeared healthy. At the end of the feeding portion of the experiments, we selected the worms randomly from each tray for the toxicity test for experiment 1. Because size as well as nutritional history may have an effect on mortality, in experiment two, we attempted to select a group of worms of similar size from each diet. The group of worms fed prawn flakes had individuals that were considerably larger (visually) than any of the worms fed E. clathrata. For the toxicity test, we selected a group of worms fed prawn flakes that were similar in size to those fed E. clathrata. This group was labelled "small". A second group of prawn flake-fed worms, the "large" worms, consisted of the larger worms in the tray. In the second experiment, the worms for each diet were selected all out of one tray, although there were two trays per diet. A subsample of worms (15) was taken from each diet treatment to determine dry weights.

For the toxicity portion of each experiment, worms were placed into the exposure cups, one worm per cup, and fed the appropriate diet. In

experiment 2, 210 mg (dry weight) of muffled (500°C for 6 hours) beach sand was added to each cup. This amount of sand did not cover the bottom of the cup, but merely provided a small amount of material with which the worm could construct a tube. The sand was added so that the worms would use this material rather than the food to construct their tubes. The worms were allowed time in the cups (24 hours for experiment 1 and one week for experiment 2) in clean, flowing seawater to establish tubes. The longer time for tube-building activity in experiment 2 was not planned, but was the result of some problems with setting up the dosing system.

The individual exposure cups used in experiment 1 were Nalgene cups (70 mm high X 62 mm diameter) with two holes (22 mm diameter) positioned on opposite sides, 1.3 cm from the bottom of the cup. The holes were covered with nylon screen, 210 μm mesh, glued with silicone sealant. The exposure cups used in experiment 2 were constructed by gluing (with silicone sealant) a 80 mm high nylon screen (210 μm mesh) cylinder into a glass petri dish (50 mm diameter). Experiments 1 and 2 were conducted more than a year apart, so differences in the exposure system represent changes made in this time period for a variety of reasons, and do not necessarily indicate modifications made based on the results of experiment 1.

The exposure cups (15 and 17 cups per box in experiments 1 and 2, respectively) were placed in plexiglass boxes (33.6 X 25 X 12.5 cm) with one box per treatment. Experiment 1 had 12 treatments, three diets X four exposure concentrations (three Cu and one control), while experiment 2, had 15 treatments, two diets, one with two size groups X five exposure concentrations (four Cu and one control). The flow rate into the boxes

was 33 mℓ/min for experiment 1, and 43 mℓ/min for experiment 2. The self-starting siphon, which provided a fluctuating water level in the box to improve the exchange of water in the exposure cups, activated about once every hour.

Toxicant was metered into the inflow water by a peristaltic pump (Harvard,* Model 1203, Millis, MA) at a rate of 0.33 and 0.43 mℓ/min for experiments 1 and 2, respectively. Toxicant stocks were made from $CuCl_2$ in deionized water. The Cu concentrations in the exposure boxes were measured twice weekly with a model 403 Perkin-Elmer* atomic absorption spectrophotometer equipped with a model 2100 Perkin-Elmer* heated graphite atomizer (HGA) retrofitted with a D_2 arc background correction unit. At each sampling, 1 mℓ was taken from each exposure box, and 0.1 mℓ of concentrated HNO_3 (ultra pure) acid was added to each sample prior to HGA analysis. Copper standards were prepared in trace metal-free seawater of the same salinity and acidity as the samples. The nominal concentrations for the treatments were 32, 56 and 100 µg/L Cu for experiment 1, and 56, 100, 180, and 320 µg/L Cu for experiment 2. The seawater used was pumped from Narragansett Bay and sand filtered (15 µm). Temperature measured 19 ± 1°C and salinity was 29 ± 1°/oo during the study.

Observations were made daily during toxicity testing. All dead animals were removed. In experiment 1, the worms were fed every other day and in experiment 2, the worms were fed daily. Uneaten food was removed and the amount fed was adjusted to minimize excess food. Worms that obviously had stopped eating were not fed.

In experiment 1, worms that survived the toxicity test were analyzed for Cu body burden. These worms were rinsed in clean seawater, placed

in vials, freeze-dried, weighed, eluted with 10% nitric acid, and analyzed for Cu with a model 403 Perkin-Elmer* atomic absorption spectrophotometer equipped with a model 2100 Perkin-Elmer* heated graphite atomizer [19].

In experiment 1, samples of food were analyzed for Cu concentration after exposure to the various Cu concentrations in the exposure boxes for 48 hours. The food was rinsed with clean seawater, blotted, freeze-dried, weighed, digested with concentrated nitric acid, and analyzed for Cu by HGA.

The LC50 values were computed by the Spearman-Karber method [20] and differences in these values were tested using the Bonferroni test [21]. Mean measured Cu concentrations of the treatments were used to calculate LC50 values. Differences in body weights and Cu concentration of worms and food were tested by one-way analysis of variance with individual comparisons made by the Tukey-Kramer multiple comparison procedure [22]. All reported differences are significant at $\alpha = 0.05$ unless otherwise stated.

Results

Diet experiment:

Of the 19 diets tested, there was no one diet that produced significantly better growth. There was no statistical different ($\alpha = 0.05$) in dry weights of worms fed 12 of the 19 diets. The lack of statistical differences was probably due to the large variation in dry weights of worms within diet treatments. Of the diets used in the toxicity tests, four of them, prawn flakes, E. siliculosus, TetraMarin, and cultured fresh E. clathrata, ranked in the top 12 (see Table 1). These diets were

chosen for use in the toxicity studies because the commericial diets, TetraMarin and prawn flakes, were easy to obtain, and the algae were cultured in our laboratory. Field-collected dried E. clathrata ranked 18th for growth in the diet experiment, and was used in the toxicity studies as a diet that yielded poor growth.

Although prawn flakes yielded the best growth, survival and tube production was better with the algal diets (Table 1). Tube production with TetraMarin, the other flake diet, was also not as good as with the algal diets. Because tube production was poor with these flake diets, we combined them with either algae (experiment 1) or sand (experiment 2) in the toxicity studies so that the worms would have enough material to build tubes. Subsequent diet studies (Schauer and Pesch, unreported data) showed that survival of worms fed prawn flakes in combination with algae or sand (for tube construction) was similar to survival of worms fed the algal diets.

Toxicity Test 1:

The initial mean dry weight of larvae was 0.05 ± 0.09 mg (n = 19). After 30 days on the various diets, worms fed the combination diet of Ectocarpus siliculosus and TetraMarin were significantly larger (7.28 ± 3.59 mg dry weight) than those fed fresh Enteromorpha clathrata (3.28 ± 2.10 mg) or field-collected dried E. clathrata (2.02 ± 1.35 mg).

The mean measured Cu concentrations in the exposure boxes were 33 ± 2, 57 ± 3, and 98 ± 5 µg/L Cu. Cu concentrations in the control treatments were 2 ± 1 µg/L Cu.

The LC50 values (Table 2) showed that there was no difference in mortality between worms fed the fresh and dried E. clathrata for an acute exposure (day 4, 7 and 10) to Cu. The acute LC50 values (day 4, 7 and 10) for worms fed the combination diet of E. siliculosus and TetraMarin could not be calculated, because mortality was less than 50% in each concentration tested. However, a comparison of the mortality on day 10 at the highest concentration, 98 µg/L Cu, showed that the worms fed the combination diet (20% dead) were more resistant to Cu during an acute exposure than worms fed the other two diets (80 and 70% dead for the fresh and dried E. clathrata, respectively). There was 100% survival in all control treatments.

The lethal response to a longer exposure (28 days) to Cu showed that the worms fed dried E. clathrata were twice as resistant as those fed fresh E. clathrata (Table 2). This difference is statistically significant because the 95% confidence intervals do not overlap. The 28-day LC50 value for the combination diet still could not be calculated (there were 33% dead at the highest Cu concentration tested). The incipient LC50 values for worms fed fresh and dried E. clathrata were estimated to be < 33 and 80 µg/L Cu, respectively. We could not estimate a more accurate incipient LC50 value for worms fed fresh E. clathrata, because we had no Cu-exposed treatment at which there was less than 50% mortality.

Beyond day 28, mortality of worms fed the combination diet did not show a dose response, the percent dead at 57 µg/L Cu were similar and sometimes greater (by a maximum of 13%, or 2 worms) than the percent dead at 98 µg/L Cu. For this reason we have not reported LC50 data beyond 28 days for the combination diet. Dissolved oxygen concentration

was lower in cups receiving the combination diet when the TetraMarin portion of the diet was not entirely consumed after 2 days. Dissolved oxygen concentrations in the cups receiving the other two diets were not depressed.

All of the Cu-exposed worms that survived the test appeared stippled with green. The green stippling was located around the edges of the parapodia and on the edges of the peristomium and prostomium. There was a distinct gradient in the worms exposed to the various concentrations of Cu; those exposed to 33 µg/L Cu had some green stippling around the parapodia, those exposed to 57 µg/L Cu had more extensive areas of green stippling, and those exposed to 98 µg/L Cu had the most extensive areas of green stippling on the parapodia, prostomium and peristomium. None of the control worms had this green stippling. The extent of the green stippling correlated with the amount of Cu found in these worms. The mean Cu concentrations (dry weight) in the worms fed the dried E. clathrata at the end of the 53-day exposure were as follows: controls, 28.5 ± 8.2 µg/gm Cu (n = 14); exposure to 33 µg/L Cu in seawater, 1958 ± 310 µg/gm Cu (n = 15); exposure to 57 µg/L Cu, 4130 ± 703 µg/gm Cu (n = 13); and exposure to 98 µg/L Cu, 9008 ± 1753 µg/gm Cu (n = 3).

The Cu concentrations in food after 48 hours in the exposure system are shown in Figure 1. These concentrations represent the maximum Cu concentration the worms could be exposed to through diet. Food eaten sooner would probably have had a lower Cu concentration. The mean Cu concentration of the TetraMarin was similar to that of the dried E. clathrata, however, the mean Cu concentrations of the two fresh algae were considerably higher. The mean Cu concentration of the fresh E. clathrata was 5 to 6-times higher than the mean Cu concentration in the

dried E. clathrata after 48 hours in all the Cu concentrations. Therefore, the worms that ate the fresh E. clathrata were probably exposed to elevated concentrations of Cu through the food as well as water. The mean Cu concentration of the fresh E. siliculosus was 4.5 and 5-times higher than the dried E. clathrata at 33 and 57 µg/L Cu, respectively, and 3.4-times higher at 98 µg/L Cu. It is uncertain whether worms fed the combination diet of fresh E. siliculosus and TetraMarin were exposed to elevated concentrations of Cu through the food, because much of the brown alga was used for tube construction. It is not known how much of the alga the worms actually ate, they may have eaten just the TetraMarin.

Based on the results of experiment 1, the rationale for diet selection and feeding protocol was changed for the next experiment. Because the fresh algae took up copper, it was decided that these were not suitable diets to feed during toxicity tests. A combination diet (more than one food) was also eliminated because of the difficulty in determining what portion was being eaten and what was used for tube construction. The feeding schedule was changed to daily to minimize the amount of time the food was in contact with the dosed seawater before it was eaten. Also, a daily feeding schedule permitted us to feed less at each feeding and thus eliminated the dissolved oxygen problem.

Toxicity test 2:

The initial mean dry weight of larvae was 0.10 ± 0.09 mg. After 27 days, the worms fed prawn flakes were larger than those fed dried E. clathrata (Table 3). The attempt to divide the worms fed prawn flakes into two size groups, with the "small" worms about the same size as those fed the dried E. clathrata, was not successful. Although, these

worms looked about the same size, the dry weight data revealed that the prawn flake-fed "small" (PFS) worms were significantly larger than the worms fed dried E. clathrata (DE) (Table 3). Also, the PFS worms were not significantly smaller than the prawn flake-fed "large" (PFL) worms at $\alpha = 0.05$. (They were significantly smaller at $\alpha = 0.10$.) At the end of the 28-day toxicity test, there was no difference in size between the surviving PFS and PFL worms in each treatment (Table 3). However, the surviving DE worms were significantly smaller than the surviving PFS and PFL worms in all treatments.

Mean measured Cu concentrations in the exposure boxes over the course of the experiment were 67 ± 6, 119 ± 7, 214 ± 10 and 380 ± 22 µg/L. Mean Cu concentration in the control treatments was 3 ± 1 µg/L.

The toxicity data (Table 4) for the short-term response, 4 to 10 days, showed that the worms fed the dried E. clathrata were significantly more sensitive to Cu than the PFS worms, which were the most resistant, and were also significantly more sensitive than the PFL worms for days 7 and 10. Comparison of the LC50 values for PFS and DE worms represent a 1.39 to a 1.98-fold difference in the acute response. The PFS worms were significantly more resistant than the PFL worms for day 7 and 10 (Table 4). In the long-term, 28 days, the LC50 values for worms fed the three diets are the same. Incipient LC50 values could not be calculated because the mortality had not reached a threshold (a number of the animals appeared near death at our lowest Cu concentration at 28 days). We decided not to continue the toxicity test because there would not be any test concentration at which mortality was less than 50%, and LC50 values calculated from this type of data would not be very accurate.

The Cu-exposed worms left alive at the end of the experiment all had the green stippling described in experiment 1. The worms exposed to 119 µg/L Cu had more extensive areas of the green stippling than those exposed to 67 µg/L Cu. Copper body burden analysis was not done on these worms.

Discussion

The results of the first toxicity experiment showed that diet affected survival of N. arenaceodentata exposed to Cu. However, the effect of diet on the toxic response probably resulted from several factors, not just the nutritional state of the test animals. Our results showed that live algae took up Cu and may have provided some worms with an additional exposure to Cu through the food. The worms fed one of the live algal diets, fresh cultured E. clathrata, when exposed to Cu were the least resistant, although fresh E. clathrata had been considered a better diet than the dried field-collected E. clathrata. In the diet experiment, N. arenaceodentata fed fresh cultured E. clathrata were 2.3-fold larger (dry wt) than worms fed dried field-collected E. clathrata; however, in the first toxicity study, worms fed these two diets were similar in weight. We can't explain this difference in growth. The worms fed the combination diet (E. siliculosus and TetraMarin) grew the best and were the most resistant in the first 28 days of the test, but their response then became obscured because of unanticipated problems (lowered dissolved oxygen in some exposure cups, and a possible difference in the amount of fresh algae consumed, therefore, a difference in amount of Cu exposure through the food). Our results indicated that a suitable diet and feeding protocol for culture conditions

is not necessarily an appropriate diet and protocol under toxicological testing conditions.

A previous study [23] has shown that diet affected the results of a toxicity test, but the effect was not related to the nutritional value of the food. Barera and Adams [23] showed that food type and concentration affected LC50 values of a toxicity test with Daphnia magna. They assumed the reduction in toxicity at increased concentration of food to be due to an adsorption of toxicant on the food. Although their results differ from ours, both studies indicate that food-toxicant interactions can affect toxicity test survival.

In the second toxicity experiment, diet had the greatest effect on mortality of N. arenaceodentata during acute exposures up to (10-day) to Cu. The worms fed dried E. clathrata (a known poor diet) were less resistant than those fed prawn flakes (a better diet as shown by the diet experiment). However, the worms fed dried E. clathrata were smaller than the prawn flake-fed "small" worms, so it is not clear if nutritional state and/or size accounted for the difference in toxicity. Traditionally, younger organisms (and, therefore, smaller) are thought to be more sensitive to toxicants. However, the toxicity data for the PFS and PFL worms indicated that the smaller worms were more resistant for these size ranges. Also, Rossi and Anderson [10] showed that juvenile Neanthes arenaceodentata exposed to the water soluble fraction of No. 2 Fuel Oil and South Louisiana crude oil were more resistant than adults. Therefore, it is likely that the lowered resistance of the worms fed dried E. clathrata is caused by nutritional differences and not just size difference. However, this remains to be tested.

No differences in LC50 values were seen among the diet treatments for the long-term (28 days) response to Cu in the second toxicity experiment. One possible explanation for a similar mortality at 28 days may be the fact that N. arenaceodentata are capable of adapting to Cu [24]. The long-term toxic response may be similar as long as the diet provides enough reserves for the animal to adapt and detoxify the Cu.

How does the influence of diet on toxicological response of N. arenaceodentata compare with the influence of other experimental variables, for example, experimental apparatus, handling, source of animals, source of seawater, etc.? The variability in LC50 values produced in an interlaboratory comparison (six laboratories, two toxicants) of a 28-day toxicity test with N. arenaceodentata ranged from a 1.18-fold difference (96 hr LC50 values for endosulfan) to a 3.31-fold difference (10-day LC50 values for silver) [1]. All the participants in this interlaboratory comparison fed their worms dried Enteromorpha sp. The differences between the 4, 7, and 10-day LC50 values of worms fed the different diets in the second toxicity experiment was within the variability measured in the interlaboratory comparison; therefore, variability due to diet in the present report was less than the variability due to other factors (experimental apparatus, handling, laboratory, source of seawater, source of animals) included in that interlaboratory comparison.

Other researchers have reported the effect of diet on toxicity test results that range from no effect to a 16-fold difference in LC50 values. Mehrle et al. [3] reported differences in 96-hr LC50 values that ranged from 2.4 to 5.7-fold difference in tests with rainbow trout fed six different diets then exposed to chlordane. In chronic studies (30 days)

with rainbow trout and bluegills exposed to Aroclor 1254, Mehrle et al. [4] showed that both species fed Oregon Test Diet were more resistant in both the acute and chronic response than fish fed Cortland No. 7 diet; with rainbow trout, the difference in 10-day LC50 values was about 16-fold, and with bluegills, the differences in 15-day and 25-day LC50 values were about 1.5 and 2.5-fold, respectively. Marking et al. [25] tested the acute toxicity of 11 chemicals to rainbow trout fry that had been reared on one of five diets. The high to low ratio of LC50 values for the diets ranged from 1.21 to 2.12 for the 11 chemicals tested. They concluded that "diet appears to have little influence on the sensitivity of young rainbow trout to chemicals in acute toxicity tests." Bengtson et al. [7] showed that the effect of diet on toxicological response differed for three marine species. The 48-hour LC50 values for mysids fed two different geographical strains of Artemia (known to differ in food quality) then exposed to Cd differed by a factor of 1.53. A chronic toxicity test with mysids could not be completed because the mysids fed one of the strains of Artemia did not survive long enough. The 48-hour and 96-hour LC50 values for Atlantic silversides fed the two strains of Artemia then exposed to Cd differed by a factor of 4 and 2.5, respectively. With sheepshead minnow, generally a very resistant species in toxicity tests, there was no difference in the 96 hour LC50 values with fish fed the different Artemia strains then exposed to the pesticide Endosulfan.

In summary, diet did affect some of the results of toxicity tests with N. arenaceodentata. However, some effects were not due just to the nutritional value of the diet; in the first experiment with live algal diets, the diet-toxicant interaction probably influenced the results.

In the second experiment, where the diet-toxicant interaction was minimized, diet had an effect on the short-term results (4 to 10 day LC50 values) but not on the long-term (28 day LC50 values). However, the difference seen between the 4, 7, and 10 day LC50 values for the different diets ranged from 1.39 to 1.98 times, which was within the range of variability (1.18 to 3.31) measured in an interlaboratory comparison with this species.

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Table 1: Diet experiment: Dry weight, survival, and number of tubes per worm for larval *Neanthes arenaceodentata* fed various diets for 28 days.

Diet	% Survival[a]	Dry weight (mg)[b] $\bar{x}$	Dry weight (mg)[b] SD	Number of tubes per worm[c]	Rank[e]
Prawn flakes	56	5.7	4.3	0.6	1
E. siliculosus (cult, fr)[d]	88	4.3	3.0	1.0	6
TetraMarin	88	4.1	3.4	0.7	7
E. clathrata (cult, fr)[d]	84	3.9	4.4	1.1	9
E. clathrata (fc, d)[d]	92	1.7	1.2	1.2	18

[a] Initially 25 worms per treatment.

[b] Initial dry weight of worms: $\bar{x}$ = 0.09 mg, SD = 0.08 mg (n = 17).

[c] Calculated by averaging the ratio of tubes to worms for days 12, 21, and 28 of the experiment.

[d] Algae: cult = cultured, fr = fresh, fc = field-collected, d = dried, fed rehydrated.

[e] Ranking according to dry weight out of 19 total diets tested.

Table 2: Experiment 1: LC50 values in µg/L Cu (measured concentration) for toxicity tests with Neanthes arenaceodentata fed three diets prior to and during tests.

	LC50 values (95% confidence limits)			
DAY	E. siliculosus and TetraMarin	Fresh E. clathrata (FE)	Dried E. clathrata (DE)	Ratio of LC50 values DE/FE
4	> 98	> 98	> 98	-
7	> 98	93 (81-106)	89 (78-102)	0.96
10	> 98	83 (74-93)	86 (77-97)	1.04
28	> 98	39 (34-44)	80 (70-91)	2.05

Table 3: Experiment 2: Weights (mg, dry) of Neanthes arenaceodentata, fed different diets, at the beginning and end (day 28) of a toxicity test with Cu (µg/L, measured concentrations).

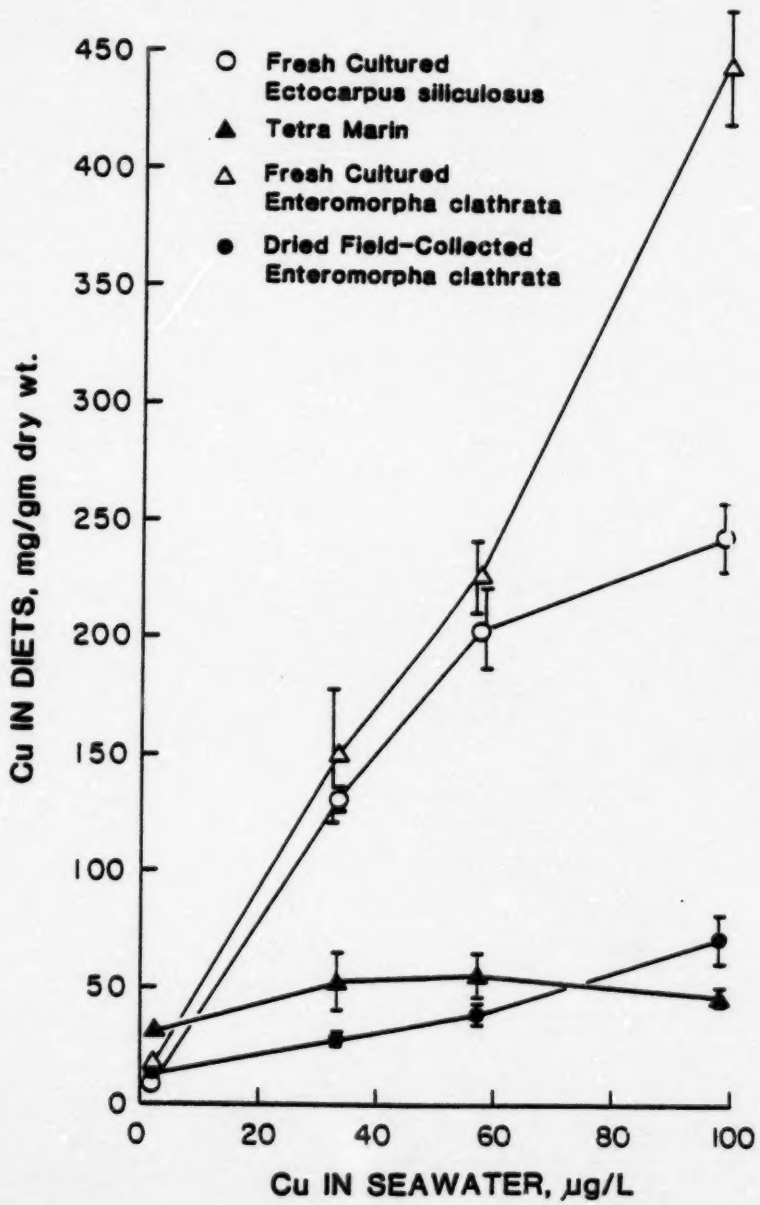
		Day 28		
DIET	Day 0[a] $\bar{x}$ ± SD (n)	Control $\bar{x}$ ± SD (n)	67 µg/L Cu $\bar{x}$ ± SD (n)	119 µg/L Cu $\bar{x}$ ± SD (n)
Prawn flakes, "large" worms	2.53 ± 0.82 (15)	25.24 ± 5.93 (17)	9.86 ± 3.89 (5)	7.14 ± 1.41 (2)
Prawn flakes, "small" worms	1.90 ± 1.01 (15)	26.29 ± 7.31 (17)	7.79 ± 2.26 (5)	7.63 ± 0.28 (2)
Dried E. clathrata	0.70 ± 0.19 (15)	11.35 ± 2.20 (17)	3.78 ± 1.34 (9)	2.73 ± 0.35 (2)

[a] Day 0 of toxicity test. Larvae at beginning of the four week feeding period prior to the toxicity test weighed $\bar{x}$ = 0.10 ± 0.09 mg (n = 25).

Table 4: Experiment 2: LC50 values (µg/L) for Neanthes arenaceodentata fed three different diets before and during a 28-day exposure to Cu. LC50 values not grouped by a line underneath are significantly different.

Day	LC50 values (95% confidence limits)			Ratios of LC50 values	
	Prawn flakes, "small" worms (PFS)	Prawn flakes, "large" worms (PFL)	Dried E. clathrata (DE)	PFS / DE	PFS / PFL
4	311 (263-368)	252 (210-302)	224 (188-267)	1.39	1.23
7	263 (232-297)	194 (161-233)	148 (135-162)	1.78	1.36
10	246 (214-282)	174 (146-208)	124 (108-143)	1.98	1.41
28	83 (68-102)	81 (68-96)	86 (74-99)	0.96	1.02

Figure 1. Experiment 1: Cu concentration (mg/gm, dry weight) in the diet components after 48 hours in the exposure system used for the toxicity test with Neanthes arenaceodentata. Each symbol indicates the mean of three samples, the bars indicate one standard deviation.


Fresh Cultured
Ectocarpus siliculosus
Tetra Marin
Fresh Cultured
Enteromorpha clathrata
Dried Field-Collected
Enteromorpha clathrata
Cu IN DIETS, mg/gm dry wt.
450
400
350
300
250
200
150
100
50
0
0
20
40
60
80
100
Cu IN SEAWATER, µg/L